REMARKS

Upon entry of these amendments, claims 1, 10, 24 and 37 are pending in this application. Support for these amendments is found throughout the specification as filed. Thus, no new matter has been added.

Oath and Declaration

The Examiner has asserted that the oath and declaration is defective as non-initialed and/or non-dated alteration has been made to the specification Applicant have corrected these deficiencies and enclosed a copy of the Oath and Declaration.

Specification

The Examiner has object to the specification because of various informalities. First, the Examiner has objected to the description of Figures 1-7, 12, 13, 17, 20, 23 and 25-32 as they contained multiple panels that are not described. Applicants have amended the brief description of the drawings to describe each panel separately.

Second, the Examiner objected to the specification because abbreviation were used without definition, for example IFNγ and TNFα. Both IFNγ and TNFα are standard abbreviations that are know to those skilled in the art as interferon gamma and tumor necrosis factor alpha. As these abbreviations are common place Applicants believe no definition of these terms are necessary. Moreover, the abbreviation "IFNγ "is defined in the specification as interferon gamma at least at page 5, line 15 of the specification as filed.

Third, the Examiner has objected to the statement regarding OX-43 expression in aortic EC does not agree with the data in Table 1. Applicants respectfully disagree. Applicants assert that the statement on regarding OX-43 expression agree with the data in Table 1. Applicants request that this objection is withdrawn.

Forth, the Examiner is unclear to the meaning if the phrase "<<face-on>>" on page 26, line 15 of the specification. The insertion of the phrase "<<face-on>>" was a typographically error. Accordingly the specification has been amended to delete the phrase. Applicants request that this objection is withdrawn.

Fifth, the Examiner objected to the specification for failing to provide proper antecedent basis for the claimed subject matter of claims 17 and 18. Claims 17 and 18 have been canceled. Applicants request that this objection is withdrawn.

Claim Objections

The Examiner has objected to claims 8 and 33 because the cell lines recited do not correspond exactly to the names of the cell lines deposited in the CNCM. Claims 8 and 33 have been canceled.

The Examiner has objected to claims 10, 11 and 18 as they recite abbreviations without providing definitions. Claim 10 has been amended to define the abbreviation "hRPE" as human retinal pigment epithelial. Claims 11 and 18 have been canceled.

The Examiner has objected to claim 19, as the preposition "in" is missing in the phase "introducing to the cell". Claim 19 has been canceled.

The Examiner has objected to claim 20, step (a) line 2 because the word selected is misspelled. Claim 20 has been canceled.

The Examiner has objected to claim 35, because it has two periods at the end of line 2 and one at the end of line 5. Claim 35 has been canceled.

Double Patenting

The Examiner has rejected claim 8 under 35 U.S.C. 101 as claiming the same invention as that of claim 2 of U.S. Patent No. 6, 183,735 B1. Claim 2 has been canceled.

The Examiner has rejected claims 1, 16, 19, 20, 25, 26, 31 and 32 under the doctrine of obviousness-type double patenting as being unpatentable over claims 1, 3, 4, 9-11 and 13 of U.S. Patent No 6,183,735 B1. Claim 1 has been amended. Claims 6, 19, 20, 25, 26, 31 and 32 have been canceled.

The Examiner has rejected claims 1, 3-6, 9 and 12-15 under the doctrine of obviousness-type double patenting as being unpatentable over claims 1, 3-7 , 9 and 10 of U.S. Patent No 6,090,624. Claim 1 has been amended. Claims 3-6, 9 and 12-15 have been canceled.

Claim Rejections -- 35 U.S.C. § 112

Claims 1, 7, 9-31 and 35 have been rejected under 35 U.S.C. § 112, first paragraph, containing subject matter not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors at the time the application was filed had possession of the claimed invention. Claims 7, 9, 23, 35-31 and 35 have been canceled. Claims 1, 10 and 24 are pending.

Claim 1 is drawn to a cell line wherein the cells of the cell line may be selected from "cell subjected to a spontaneous genetic modification leading to an extended life span" or " cells comprising a sequence able to activate the endogenous hTRT gene". According to the Examiner, the specification does not describe in detail such cells or details of the genetic modification or any sequence able to activate an endogenous hTRT genes or cell containing such sequence. The claim have been amended delete the phrases "cell subjected to a spontaneous genetic modification leading to an extended life span" and "cells comprising a sequence able to activate the endogenous hTRT gene". This rejection should be withdrawn.

Claim 10 is drawn to specific human retinal pigment epithelial cell lines. The Examiner requests a biological deposit of the recited cell lines. Applicants will deposit the recited cell lines upon an indication of allowable subject matter.

Claim 24 is drawn to a method of using cells subjected to a spontaneous genetic modification leading to an extended lifespan. According to the Examiner the specification does not describe in detail such cells or the details of the genetic modification. Claim 24 has been amended to delete the phrase "cells subjected to a spontaneous genetic modification leading to an extended lifespan". This rejection should be withdrawn. In addition, according the Examiner, claim 24 does not reasonably provide enablement for a method of producing a polypeptide *in vivo* in a mammalian host. Applicants respectfully disagree. The Examiner on his own admission states that the specification is enabling for method of producing a polypeptide *in vitro*. (See, Office Action Mailed March 28, 2001, page 16, paragraph 14). Such an enablement is all that is required as a matter of law. Furthermore, the Examiner asserts that the specification is not enabled for methods of gene therapy. Applicants assert that the method of claim 24 is *not* gene therapy, but a method of cell transplantation. Claim 24 requires taking a cell that has been

transformed to produce a polypeptide and culturing the cell in a biological compatable medium including the subretinal space of a mammal. The specification provides numorous examples detailing the method of transplanting epithelial cells to the restinae of rats. See for example, EXAMPLE 10, page 38 lines 10-20 and EXAMPLE 11, page 43, line 24-28.

Claims 1-35 are rejected under 35 USC 112, second paragraph as being indefinite for failing to particularly point out and distinctly claims the subject matter which the Applicant regard as the invention. Claim 2-9, 11-23 and 25-35 have been canceled. Claim 1, 10 and 24 are pending.

According to the Examiner, claim 1 is vague and indefinite because the Markush group has only one member. Claim 1 has been amended to include more than one member in the Markush group. This rejection should be withdrawn.

According to the Examiner, the limitation in claim 1, "a sequence able to activate the endogenous hTRT " is vague and indefinite. The phase a sequence able to activate the endogenous hTRT " had been deleted. This rejection should be withdrawn.

According to the Examiner, claim 1 is vague and indefinite because there is insufficient antecedent basis for the limitation "the human telomerase reverse transcriptase gene" . The phrase "the human telomerase reverse transcriptase gene" has been deleted. This rejection should be withdrawn.

According to the Examiner, claim 24 is vague and indefinite because it recites the limitation " wherein the cells are subjected to a spontaneous genetic modification". The phrase "wherein the cells are subjected to a spontaneous genetic modification" has been deleted. This rejection should be withdrawn.

Claim Rejections -- 35 U.S.C. § 102

Claim 1, 9, 12, 13, 15 and 19 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Bodnar *et al* Science (1998) 279:349-352 ("Bodnar"). According to the Examiner, Bodnar teaches a human retinal pigment epithelial cell line comprising a recombinant polynucleotide comprising the human telomerase reverse transcriptase gene. Claims 9, 12, 13,15 and 19 have been canceled. Claim 1 has been amended to delete the limitation of comprising the

human telomerase reverse transcriptase gene or a sequence able to activate the endogenous genes. This rejection should be withdrawn.

Claims 1, 9 and 11 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Dunn *et al* Exp. Eye Res (1996) 62: 155-169 ("Dunn"). Dunn teaches a human retinal pigment epithelial cell line (ARPE-19) which arose spontaneously having increase growth potential and a normal karyotype. Claims 9 and 11 have been canceled. Claim 1 has been amended to require that the cell of the invention further be transformed with an expression vector comprising a polynucleotide coding for a polypepeptide such as BDNF, NT-4, or CNTF. In contrast, the cell taught by Dunn, are *not* transformed (See, March 28, 2001 Office Action,, page 20, paragraph 19). Dunn does not teach or suggest a human retinal pigment epithelial cell transformed with a polypeptide and certainly not the specifically recited group of peptides required by amended claim 1. This rejection should be withdrawn.

Claims 1,-6, 9 and 19 have been rejected under 35 U.S.C. § 102(b) as being anticipated by Dutt *et al* Oncogene (1990) 5: 195-200 ("Dutt"). Dutt teaches a human retinal pigment epithelial cell line comprising a recombinant polynucleotide comprising an oncogene. Claim 2-6, 9 and 19 have been canceled. Claim 1 has been amended to delete the limitation of cell comprising a recombinant polynucleotide comprising an oncogene, the claim recited three specific human cell lines two of which the Examiner has acknowledged are free of prior art (hRPE-1 and hRPE-116. The rejection should be withdrawn.

Claim 35 has been rejected under 35 U.S.C. § 102(b) as being anticipated by Manuelli *et al.* Clam 35 has been canceled. This rejection should be withdrawn.

Claim Rejections -- 35 U.S.C. § 103

Claims 1, 9, 12-16, 19 and 20 have been rejected under 35 USC 103 (a) as being unpatentable over Bodnar. Claims 9, 12-16, 19 and 20 have been canceled.

Claims 1,9 and 12-21 have been rejected under 35 USC 103 (b) as being unpatentable over Bodnar in view of Litchfiled et al. Claims 9 and 12-21 have been canceled.

Claims 1-6, 9, 19 and 36 have been rejected under 35 USC 103 (b) as being unpatentable over Dutt. Claims 2-6, 9, 19 and 36 have been canceled.

Claim 1 is pending. Applicants have amended claim 1, to incorporate the limitation of claims 10 and 11. The Examiner concluded that claim 10 was free of prior art thus with respect to cells lines hRPE-7 and hRPE-116 the subject matter of claim 10, Bodnar, Dutt, and Litchefiled are not applicable. With respect to cell line APRE-19, Bodner does not teach nor suggest an APRE-19 cell transformed to produce a polypeptide such DNF, NT-4, or CNTF.

Litchefield, teaches the use of cytokines as therpeutic factors for degenerative retinal disease. Litchefield neither alone or combined with Bodnar teaches or suggests a APRE-19 cell line transformed to produce a polypeptide.

Dutt does not teach a method for making a retinal epithial cell line, Dutt merely *suggests* that their method may be useful to *try* in establishing a cell line from ocular tissue. The teachings of Dutt does not provide a reasonable expectation of success in the production of a retinal epithelial cell line. Moreover, Dutt fails to teach or suggest the cell of the present invention which is a rat retinal pigment epithelial cell that is transformed to produce a polypeptide.

Version with Markings to Show Changes Made

In the Specification:

The section Brief Description of the Drawing beginning on page 4 of the specification has been amended as follows:

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 A is a ~~set of micrographs~~photomicrograph showing the morphology of the primary cultures of retinal endothelial cells~~(~~.

FIG. 1~~A),~~B is a photomicrograph showing the morphology of the primary cultures of retinal pigment epithelial cells ~~(FIG. 1B) and the IO/JG2/1 clones (FIG. 1C) and IO/LD7/4 clones (FIG. 1D) according to the invention.~~.

~~FIG. 2 is a set of micrographs showing transmission electron micrographs of the IO/LD7/4 cells (FIG. 2A) and IO/JG2/1 cells (FIG. 2B; FIG. 2C).~~

~~FIG. 3 is a set of micrographs showing the nuclear staining obtained in the presence of antibodies directed against the T-antigen, in IO/JG2/1 (FIG. 3A) and IO/LD7/4 (FIG. 3B).~~

~~FIG. 4 is a set of graphs showing the expression of different endothelial markers in the IO/JG2/1 cultures (FIGS. 4A-4F).~~

~~FIG. 5 is a set of graphs showing the comparative expression of different epithelial markers in~~FIG. 1C is a photomicrograph showing the morphology of the primary cultures ~~(FIG. 5A, FIG. 5B) and~~of the IO/~~LD7~~JG2/4 ~~clone (FIG. 5C, FIG. 5D).~~ 1 clones.

~~FIG. 6 is a set of graphs showing the expression of the adhesion molecule ICAM-1 (FIG. 6A, FIG. 6B) and the antigens of the class I (FIG. 6C, FIG. 6D) and class II (FIG. 6E, FIG. 6F) major histocompatibility complex in the IO/LD7/4 clone in the absence (FIG. 6A, FIG. 6C, and FIG. 6E) or presence (FIG. 6B, FIG. 6D, and FIG. 6F) of induction by interferon gamma (IFNγ).~~

FIG. 1D is a photomicrograph showing the morphology of the primary cultures of IO/LD7/4 clones.

FIG. 2A is a transmission electron micrographs of the IO/LD7/4 cells.

FIG. 2B is a transmission electron micrographs of the IO/JG2/1 cells.

FIG. 2C is a transmission electron micrographs of the IO/JG2/1 cells.

FIG. 3A is a micrograph showing the nuclear staining obtained in the presence of antibodies directed against the T-antigen in IO/JG2/1 cells.

FIG. 3B is a micrograph showing the nuclear staining obtained in the presence of antibodies directed against the T-antigen in IO/LD7/4 cells

FIG. 4A is a graph showing the expression of endothelial marker, Griffonia in the IO/JG2/1 cultures.

FIG. 4B is a graph showing the expression of endothelial marker, ICAM-1 in the IO/JG2/1 cultures.

FIG. 4C is a graph showing the expression of endothelial marker, RECA-1 in the IO/JG2/1 cultures.

FIG. 4D is a graph showing the expression of endothelial marker, OX-18 in the IO/JG2/1 cultures.

FIG. 4E is a graph showing the expression of endothelial marker, OX-6 in the IO/JG2/1 cultures.

FIG. 4F is a graph showing the expression of endothelial marker, OX-17 in the IO/JG2/1 cultures.

FIG. 5A a graphs showing the comparative expression of epithelial marker, cytokeratin in the primary cultures.

FIG. 5B a graphs showing the comparative expression of epithelial marker, RET-PE2 in the primary cultures.

FIG. 5C a graphs showing the comparative expression of epithelial marker, cytokeratin in the IO/LD7/4 clone.

FIG. 5D a graphs showing the comparative expression of epithelial marker, RET-PE2 in the IO/LD7/4 clone.

FIG. 6A is a graph showing the expression of the adhesion molecule ICAM-1 in the IO/LD7/4 clone in the absence of induction by interferon gamma (IFNγ).

FIG. 6B is a graph showing the expression of the adhesion molecule ICAM-1 in the IO/LD7/4 clone in the presence of induction by interferon gamma (IFNγ).

FIG. 6C is a graph showing the expression of the adhesion molecule OX-18 in the IO/LD7/4 clone in the absence of induction by interferon gamma (IFNγ).

FIG. 6D is a graph showing the expression of the adhesion molecule OX-18 in the IO/LD7/4 clone in the presence of induction by interferon gamma (IFNγ).

FIG. 6E is a graph showing the expression of the adhesion molecule OX-6 in the IO/LD7/4 clone in the absence of induction by interferon gamma (IFNγ).

FIG. 6F is a graph showing the expression of the adhesion molecule OX-6 in the IO/LD7/4 clone in the presence of induction by interferon gamma (IFNγ).

FIG. 7A is a bar graph showing, for the IO/LD7/4 cells, the expression of class II I-A histocompatibility antigens (black) and I-E histocompatibility antigens (shaded) in response to IFNγ from 0 to 5 days.

FIG. 7 B is a ~~set of~~ bar ~~graphs~~graph showing, for the IO/LD7/4 cells, the expression of the adhesion molecules ~~ICAM-1 (FIG. 7B, black bars) and~~ VCAM-1 (~~FIG. 7B,~~ shaded bars) ~~and the class II I~~ICAM~~-A histocompatibility antigens~~l (~~FIG. 7A,~~ black~~) and I-E histocompatibility antigens (FIG. 7A, shaded~~ bars) in response to IFNγ from 0 to 5 days.

FIG. 8 is a bar graph showing the migration of T-lymphocytes across monolayers consisting of the primary cultures of retinal endothelial cells (REC), retinal pigment epithelial cells (RPE) or the IO/JG2/1 and IO/LD7/4 clones.

FIG. 9 is a set of electron micrographs of IO/LD7/4 cells co-cultivated with dissociated retina; the debris of external segments (ROS) is adjacent to the cells and found in the phagosomes (P).

FIG. 10 is a micrograph showing IO/LD7/4 cells cultivated on slides coated with Matrigel®. The cells show a high contractile capacity, creating stress lines in the matrix.

FIG. 11 is a micrograph showing the hexagonal morphology of the cells obtained after grafting the IO/LD7/4 cells onto the retina of Sprague-Dawley rats.

FIG. 12A is a ~~set of graphs~~graph showing the means and standard deviations of the latency times of the pupillary reflexes in response to a light stimulus in rats grafted with primary retinal pigment epithelial cells~~(~~.

FIG. 12~~A) and~~B is a graph showing the means and standard deviations of the latency times of the pupillary reflexes in response to a light stimulus in rats grafted with IO/LD7/4 cells ~~(~~.

FIG. 12~~B) and~~C is a graph showing the means and standard deviations of the latency times of the pupillary reflexes in response to a light stimulus in control animals (blank operation) ~~(FIG. 12C); the data in~~

FIG. 12D ~~show the responses of a~~is a graph showing the means and standard deviations of the latency times of the pupillary reflexes in response to a light stimulus in r dystrophic RCS rat as a function of age; ~~The~~the mean latency time of a non-dystrophic rat is 0.48 ± 0.04 second; L = left eye and R = right eye.

FIG. 13A is a ~~set of graphs~~graph showing the means and standard deviations of the amplitude of the pupillary reflex responses to light in rats grafted with primary retinal pigment epithelial cells~~(~~.

FIG. 13~~A) and~~B is a graph showing the means and standard deviations of the amplitude of the pupillary reflex responses to light in rats grafted with IO/LD7/4 cells~~(~~.

FIG. 13~~B) or~~C s a graph showing the means and standard deviations of the amplitude of the pupillary reflex responses to light in control rats ~~(FIG. 13C); the data shown in~~

FIG. 13D ~~correspond to the~~s a graph showing the means and standard deviations of the amplitude of the pupillary reflex responses ~~of~~to light in a dystrophic RCS rat as a function of age. The mean amplitude of response of a non-dystrophic 6-month-old animal is 19.7±5.7%; L = left eye and R = right eye.

FIG. 14 is a bar graph showing the modifications of the mean activity of rats placed in cages with walls of different designs; rats grafted with IO/LD7/4 cells (shaded bars), control rats (blank operation or sham; white bars); blank = plain walls; check = decorated walls.

FIG. 15 is a bar graph showing the number of active units of visual field in the superior colliculus, expressed as a percentage of the number of active units of visual field; the IO/LD7/4 and primary retinal pigment epithelial (RPE) cells are capable of slowing down the loss of visual field in the grafted animals compared with the non-grafted animals (control or sham).

FIG. 16 ~~is a representation~~A-D are representations of recordings showing, on the one hand, a 2-dimensional view of the superior colliculus (FIG. 16A and FIG. 16B, in which C = caudal, M = medial, R = rostral and L = lateral), and on the other hand the maps of the corresponding visual fields of the retina (FIG. 16C and FIG. 16D, in which D = dorsal, N = nasal, V = ventral and T = temporal). The crosses on the map of the colliculus represent the zones for which no recording could be obtained. The dots correspond to the zones for which recordings could be obtained; the left-hand FIGS. (FIG. 16A and FIG. 16C) represent the recordings of a dystrophic 6-month-old rat. The recordings could be made from a single unit (light zone), which is typical of animals of this age. The right-hand FIGS. (FIG. 16B and FIG. 16D) represent the recordings of a rat grafted with IO/LD7/4 cells on the superior temporal retina. It is observed that responses can be obtained from a wide zone of the superior colliculus.

FIG. 17A-D is a set of graphs showing some of the differences relating to the histological characteristics of the retinas of rats grafted with primary retinal pigment epithelial (RPE) cells or IO/LD7/4 cells. The number of nuclei in the outer nuclear layer is shown in FIG. 17A. The number of nuclei in the inner nuclear layer is shown in FIG. 17B. The depth of the outer plexiform layer in μm is shown in FIG. 17C. The relative zone (%) of retinas saved by grafting is shown in FIG. 17D.

FIG. 18 is an identification of the deposit of the retinal pigment epithelial cells with extended life-span called IO/LD7/4 on 18th April 1996 in the Collection Nationale de Cultures dė Micro-organismes (CNCM) held by the Institut Pasteur, 28 rue de Docteur Roux, 75724 PARIS CEDEX 15, under the identification no. I-1694. The indications in section B states (in French):

> With regard to the nominations in which a European patent is applied for, until the publication of the mention of the grant of the European patent or until the date on

which the application shall be refused or withdrawn or shall be deemed to be withdrawn, a sample of the deposited microorganism shall be available only by the issue of a sample to an expert nominated by the requester (Rule 28.4) of the EPC).

Figure 19 is an identification of the deposit of the retinal endothelial cells with extended life-span called IO/JG2/1 on 18th April 1996 in the Collection Nationale de Cultures de Micro-organismes held by the Institut Pasteur, 28 rue de Docteur Roux, 75724 PARIS CEDEX 15, under the identification no. I-1695. The indications in section B states (in French):

With regard to the nominations in which a European patent is applied for, until the publication of the mention of the grant of the European patent or until the date on which the application shall be refused or withdrawn or shall be deemed to be withdrawn, a sample of the deposited microorganism shall be available only by the issue of a sample to an expert nominated by the requester (Rule 28.4) of the EPC).

FIG. 20 A-C is a pictograph and a set of graphs showing the head tracking in RCS dystrophic (dys) and non-dystrophic rats (con) at 8 weeks (8w; FIG. 20B) and 50 weeks of age (50w; FIG. 20C). The ordinate is a measure of time spent tracking to the revolving drum (FIG. 20A).

FIG. 21 is a graph showing the tracking behavior in dystrophic RCS rats to stripes of different width. The shams perform near baseline. The cell transplanted rats show significant spared performance.

FIG. 22 is a set of recordings of elevation of luminance over baseline in a set of animals. Each record shows responses at points 200 μm apart across the superior colliculus (dorsal retinal representation above, temporal to left). Unshaded show responses equal to or less than 2.0 candela/m^2; light shading 2.1-2.9 and dark shading >2.9. Animals were tested at 5 months post operative. As can be seen, there is a small sham effect around the injection site, but significantly greater preservation after cell grafting.

FIG. 23 A-B is a set of graphs showing the head tracking after human cell line grafts at 7 weeks (FIG. 23A) and 14 weeks of age (FIG. 23B). Note that the cell transplanted dystrophic rats (cells) perform much like non-dystrophics (congenic) by 10-11 weeks (FIG. 23B) post-transplantation and are significantly improved over sham-injected rats.

FIG. 24 is a set of recordings showing the threshold responses (details as in FIG. 22) recorded 12 weeks post-operative. Here the control was a non-dystrophic that received a graft.

The sham injected animal shows evidence of remaining responsiveness seen in unoperated dystrophics around the edge of the superior colliculus. The 2 transplanted animals are examples showing substantially improved responsivenes.

FIG. 25 A-B is a set of photographs. FIG. 25A shows an aspect of the subcutaneous graft on cryostat section without any immunohistochemical treatment. The yellow/brown color is restricted to the graft zone (hRPE clone 7, 3 weeks post-implantation. Bright field, x80). FIG. 25B shows a field of the same section at high magnification. The coloration is intracytoplasmic and present in the majority of the cells (Bright field, x320).

FIG. 26 A-B is a set of photographs. FIG. 26A is a hematoxylin and eosin stain of hRPE clone 7, 3 weeks following subcutaneous post-implantation in the flank of a nude mouse (Bright field, x40). FIG. 26B is a hematoxylin ~~hexatoxylin~~ and eosin ~~Eosin~~ stain at high magnification of the same section. The colored cells show a normal structure of the nucleus. The graft is infiltrated by cells bearing elongated nuclei ~~nucleus~~, probably fibroblasts (Bright field, x800).

FIG. 27 A-B is a set of photographs showing Schmorl staining. FIG. 27A is a Schmorl stain of hRPE clone 7, 3 weeks following subcutaneous post-implantation in the flank of a nude mouse. The reaction with lipofuscins and/or melanin produce a blue/green staining of the cells within the graft site (Bright field, x160). FIG. 27B is a Schmorl stain of hRPE clone 7, 15 weeks following subcutaneous implantation. A few green/black cells are found at the graft site (Bright field, x160).

FIG. 28 A-F is an analysis of primary human RPE cells and human RPE cells with extended life-span. FIG. 28A and FIG. 28B are a set of phase contrast micrographs of (FIG. 28A) contact-inhibited monolayer of primary cultured human donor RPE cells 10 days after seeding and (FIG. 28B) of human clone hRPE7 cells derived from culture depicted in A (scale bars =100 μm). Both cultures exhibit cobblestone morphology characteristic of RPE cells. FIG. 28C shows the immunocytochemical detection by epifluorescence microscopy of SV40 large T antigen showing correct nuclear expression (scale bar = 20 μm). FIG. 28D shows the immunocytochemical detection by confocal scanning laser microscopy (projected images) of junctional protein ZO-1 showing an almost continuous pattern of peripheral staining (scale bar = 20 μm). FIG. 28E shows the immunocytochemical detection of the RPE cytokeratins 8 and 18. FIG. 28F is an overlay of images depicted in immunomicrographs D and E plus an additional bisbenzimide

DNA stain (blue) to highlight the cell nuclei (scale bar = 20 μm).

FIG. 29 A-C is a representation of head tracking to high contrast square-wave gratings. FIG. 29A is a photograph of head tracking apparatus showing RCS rat in holding chamber and rotating drum lined with square-wave grating. FIG. 29B shows the total amount of time spent tracking a moving square-wave grating in seconds over a period of 4 minutes after 10 weeks post-transplant. (FIG. 29C) Head tracking 20 weeks post-transplantation. Error bars represent s.e.m. *$p<0.01$ represents a significant difference as compared to both Sham and Dystrophic groups. $^{+}p<0.05$ represents a significant difference as compared to the hRPE7 group.

FIG. 30 A-E is a set of recordings showing the threshold light sensitivity maps of congenic, dystrophic, hRPE7 transplanted and sham operated RCS rats. RCS rats were divided into 4 groups: (FIG. 30A) normal (3 non-dystrophic rats); (FIG. 30B) no treatment (6 dystrophic rats); (FIG. 30C) hRPE7 cells injected into one eye (5 dystrophic rats); and (FIG. 30D) sham injected (5 dystrophic rats). Schematic representation of a dorsal view of the superior colliculus showing respective thresholds for 76 individual recording sites (color coded squares). A log scale of thresholds measured in candela/m^2 is shown. To test efficacy of hRPE7 grafts versus sham injected animals, significance was determined at each of the 76 points using a randomization test. An area of significantly improved visual function was recorded for the hRPE7 transplanted animals as shown in (FIG. 30E). The corresponding topological representation of the retina onto the superior colliculus is indicated by the letters D (dorsal), V (ventral), N (nasal), and T (temporal). The arrow represents the grafted quadrant.

FIG. 31A-C is a set of photographs showing anatomical changes in the photoreceptor cell layer following transplantation of hRPE7 in RCS rats. Sections of the retina from (FIG. 31A) a 6-month-old non-dystrophic RCS rat showing full outer nuclear layer thickness. (FIG. 31B) Sham operated RCS rat 5 months post operation showing complete ablation of the outer nuclear layer. (FIG. 31C) hRPE7 transplanted RCS rat 5 months post graft demonstrating significant preservation of the outer nuclear layer. Sections were stained with cresyl violet. GC: ganglion cell layer. INL: inner nuclear layer. ONL: outer nuclear layer. RPE: retinal pigment epithelial cell layer. Scale bar = 25 μm. Panel (FIG. 31A) is also stained with RT-97 anti-heavy neurofilament antibody.

FIG. 32 A-B is a set of bar graphs showing an assessment of visual function in RCS rats with the hRPE cell line 7 (passages 14 and21) as compared to those with ARPE-19. The results are obtained from a visual stimulus, which moves in an anti-clockwise direction, which viewed from the right eye is a temporal to nasal direction. All transplants were placed in the superior temporal retina of the right eye. Data represent the total time spent head tracking. Experimental groups: Con: non-dystrophic RCS rats (n=10), Dys: un-treated dystrophic RCS rats (n=10), Sham: dystrophic RCS rats received injection of 1 μl cell culture medium (used for hREP7; n=8). H1RPE7p14: hRPE7 at passage 14 injected into dystrophic RCS rats (n=10), H1RPE7p21: hRPE7 at passage 21 injected into dystrophic RCS rats (n=7), ARPE-19p22: ARPE-19 at passage 22 injected into dystrophic RCS rats (n=5). FIG. 32A shows, for comparison, data of each experimental group were pooled over all square wave gratings (0.125, 0.25 and 0.5 cycles/degree). A significant difference ($p<0.01$) is apparent between the groups which either received cell grafts or remained untreated (sham, dystrophics). ARPE-19 clearly shows a therapeutic effect which comes close to hRPE7p21. FIG. 32 B shows the total time spent head tracking at 0.125 cycles/° and 0.25cycles/° grating stimulus. While congenic animals were also able to track a grating of 0.5cycles/degree none of the other groups (including transplanted groups) were able to do so.

The paragraph on page 26 begining at line 13 as been amended as follows:

In the majority of sections, the epithelial cell layer was a single layer (monolayer), but a multilayer was observed in certain regions. When the blocks were sectioned [<<face-on>>] the cells of the invention possessed *in vivo* the hexagonal phenotypic characteristics of the primary retinal pigment epithelial cells, even though these characteristics were lost *in vitro* (FIG. 11). This result was clear when the transplant has more than one layer of cells.

In the Claims:

Claims 2-9, 11-23, and 25-36 have been canceled. Claim 37 is new.

Claim 1 has been amended as follows:

1. An injectable, non-tumorigenic, human [mammalian] retinal pigment epithelial cell line,

wherein [the cells]the cell line are is selected from the group comprising hRPE-7, hRPE-116 and ARPE-19 wherein the cells of the cell line [consisting of cell]:

[(i) comprising a recombinant polynucleotide comprising an oncogene,

(ii) subjected to a spontaneous genetic modification leading to an extended life-span, and

(iii) comprising a recombinant polynucleotide comprising the human telomerase reverse transcriptase gene (hTRT) or a sequence able to activate the endogenous hTRT gene; and]

(a) comprise an expression vector comprising a polynucleotide coding for a polypepeptide selected from the group comprising BDNF, NT-4, CNTF, Axokine, FGF-2 (bFGF), IGF I, IGF II, TGFβ-II, Midkine, IL-1β, TNF, NGF, IL-2/3, ILF, IL-6, NTN, Neublastin, VEGF, GDNF, PDGF, LEDGF and PEDF.

(b) [wherein the cells of the cell line] can non-tumorigenically interact with retinal cells of a mammalian host.

Claim 10 has been amended as follows:

10. The cell line [of claim9], [wherein the cells are of the] human retinal pigment epithelial-7 (hRPE-[cell line] 7) [or] and human retinal pigment epithelial-116 (hRPE-[cell line] 116).

Claim 24 has been amended as follows

24. A method of producing a therapeutic polypeptide to treat primary or secondary ophthalmologic or neurological disorders, comprising incubating cells of a mammalian retinal pigment epithelial cell line wherein the cell line is selected from the group comprising hRPE-7, hRPE-116 and ARPE-19 in a biological compatible medium such that the cell line produce the polypeptide

[(a)wherein the cells are subjected to a spontaneous genetic modification leading to an extended life-span;] and wherein the cells of the cell line comprise an expression vector comprising a polynucleotide coding for a polypeptide selected from the group comprising BDNF, NT-4, CNTF, Axokine, FGF-2 (bFGF), IGF I, IGF II, TGFβ-II, Midkine, IL-1β, TNF, NGF, IL-2/3, ILF, IL-6, NTN, Neublastin, VEGF, GDNF, PDGF, LEDGF and PEDF.[for treating primary or secondary ophthalmologic or neurological disorders.]

CONCLUSION

On the basis of the foregoing amendments and remarks, Applicants respectfully submit that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact either of the undersigned at the telephone number provided below.

Respectfully submitted,

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